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Global Gene Expression Profiling of the Asymptomatic Bacteriuria *Escherichia coli* Strain 83972 in the Human Urinary Tract†

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Urinary tract infections (UTIs) are an important health problem worldwide, with many million cases each year. *Escherichia coli* is the most common organism causing UTIs in humans. The asymptomatic bacteriuria *E. coli* strain 83972 is an excellent colonizer of the human urinary tract, where it causes long-term bladder colonization. The strain has been used for prophylactic purposes in patients prone to more severe and recurrent UTIs. For this study, we used DNA microarrays to monitor the expression profile of strain 83972 in the human urinary tract. Significant differences in expression levels were seen between the in vivo expression profiles of strain 83972 in three patients and the corresponding in vitro expression profiles in lab medium and human urine. The data revealed an in vivo lifestyle of microaerobic growth with respiration of nitrate coupled to degradation of sugar acids and amino acids, with no signs of attachment to host tissues. Interestingly, genes involved in NO protection and metabolism showed significant up-regulation in the patients. This is one of the first studies to address bacterial whole-genome expression in humans and the first study to investigate global gene expression of an *E. coli* strain in the human urinary tract.

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. It is estimated that there are more than 10 million annual cases in Western Europe alone. For anatomical reasons, UTIs primarily affect girls and women, and 40 to 50% of adult women have experienced at least one UTI episode (47). *Escherichia coli* is the most important etiological agent of UTIs and is responsible for >80% of all such infections. UTI usually starts as a bladder infection but often evolves to encompass the kidneys and, ultimately, can result in renal failure or dissemination to the blood. UTI is the most common infection in patients with a chronic indwelling bladder catheter; bacteriuria is essentially unavoidable in this patient group (13). Colonization of urine in the absence of clinical symptoms is called asymptomatic bacteriuria (ABU). ABU occurs in up to 6% of healthy individuals and 20% of elderly individuals. ABU strains generally do not cause symptoms, and most patients with ABU do not need treatment. Furthermore, colonizing ABU strains may actually help to prevent infections by other, more virulent bacteria (9, 18, 60, 61).

E. coli 83972 is a clinical isolate capable of long-term bladder colonization. The strain was originally isolated from a young Swedish girl with ABU who had carried it for at least 3 years without symptoms (2, 32). It is well adapted for growth in the human urinary tract (UT), where it establishes long-term bacteriuria (2, 18, 66, 68). Strain 83972 has been used for prophylactic purposes in numerous studies; as such, it has been used as an alternative treatment for patients with recurrent UTIs who are refractory to conventional therapy (9, 18). In this case, the bladders of patients are deliberately colonized with *E. coli* 83972 in order to prevent disease-causing strains from colonization. Deliberate colonization with *E. coli* 83972 has been shown, for example, to reduce the frequency of UTI in patients with spinal cord injury and neurogenic bladder (68), and the strain can prevent catheter colonization by bacterial and fungal uropathogens (9, 60, 61). In effect, extensive trials have shown that infections with potentially dangerous uropathogenic *E. coli* (UPEC) strains often do not take place in such patients as long as the ABU strain stays in the bladder. As such, the strain is probably the most extensively "field-tested" UTI strain in humans in the world.

Although *E. coli* 83972 causes little or no symptoms in patients, its molecular profile points to a pathogenic past; all available evidence suggests that the ancestor of 83972 was a pyelonephritic UPEC strain. Thus, multilocus sequence typing of 83972 shows that it belongs to the ECOR B2 clonal group (http://www.mlst.net/). *E. coli* strains belonging to group B2 are associated with pyelonephritis and other extraintestinal invasive clinical syndromes, such as bacteremia, prostatitis, and meningitis. Also, we recently characterized the *fim*, *pap*, and *foc* gene clusters of the strain and showed that although the fimbriae encoded by these clusters are nonfunctional, the strain contains sequence information that bears witness of a pathogenic past (26, 41). Thus, the *fimH* allele of 83972 encodes minor amino acid variations (compared with the K-12 version) that are consistent with those of previously characterized pyelonephritis isolates (51–53). Furthermore, the strain has a copy of the F14 *papA* variant that has been associated with other virulence factors, including S and F1C fimbriae, hemolysin, and cytotoxic necrotizing factor 1, from *E. coli* strains in phylogenetic group B2 (22). It also harbors known virulence-associated genes, such as *kps*, *iutA*, *fyuA*, and *malX* (10), which have not yet been characterized. Whether these

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are expressed and functional in the strain has yet to be examined.

Although *E. coli* 83972 carries genes belonging to several different fimbria-encoding virulence factor clusters (19), the strain has never been reported to adhere to any kind of cells originating from the human urinary tract (2, 19, 66). Recently, we found that the strain is unable to express functional type 1, P, and F1C fimbriae due to a deletion in the *fim* cluster and mutations in the *pap* and *foc* clusters (26, 41). The inability of strain 83972 to express functional type 1, P, and F1C fimbriae explains to a large degree why it does not cause symptoms in humans, because these fimbriae tend to activate antibacterial host defenses. Thus, while fimbria-assisted binding to epithelial host cells undoubtedly counteracts removal by hydrodynamic flow forces in the urinary tract, binding to host epithelial cells is often a mixed blessing for the bacteria because it frequently triggers host responses such as cytokine production, inflammation, and exfoliation of infected epithelial cells (17, 36, 46, 67).

DNA microarray-assisted functional genomics provides a genome-wide portrait of the transcriptome of an organism and discloses how a bacterium adapts to an environmental niche at the transcriptional level. Here we analyze the global expression profile of *E. coli* 83972 in the human urinary tract with a view to gaining further insight into the mechanisms by which this organism is able to grow in urine and colonize the human bladder without evoking the host response. This is the first study where a transcriptome profile of a UTI strain isolated directly from patients has been analyzed. We employed GeneChip *E. coli* Genome 2.0 arrays (Affymetrix), which include the genome of the uropathogenic strain CFT073 in addition to the MG1655 genome, to get a clearer picture of which urovirulence-associated genes *E. coli* 83972 carries and expresses during colonization in vivo.

MATERIALS AND METHODS

Bacterial strain. *E. coli* 83972 (OR:K5:H$^-$) is a prototype ABU strain and lacks defined O and K surface antigens. It belongs to ECOR group B2, together with many other UPEC strains, such as the well-characterized and virulent *E. coli* isolates CFT073, 536, and J96.

Sampling and stabilization of RNA for microarray experiments with in vivo-grown *E. coli* 83972. Three volunteers, one male and two females, all of whom were long-term carriers of ABU *E. coli* 83972, were used in the study. Urine samples from the three patients were extracted using clean-catch technique, and a 45-ml sample from each patient was immediately mixed with 2 volumes of RNAprotect bacterial reagent (QIAGEN AG, Basel, Switzerland), incubated for 5 min at room temperature to stabilize RNA, and centrifuged. The pellets were then stored at −80°C. A part of each patient's sample was extracted before RNA stabilization for investigation of adherence to human uroepithelial cells. The bacterial cells were mixed with uroepithelial cells in phosphate-buffered saline and incubated for 1 h at 37°C; the cells were washed three times in phosphate-buffered saline, and bacterial adherence was examined by light microscopy.

Growth conditions and stabilization of RNA for microarray experiments with in vitro-grown *E. coli* 83972. Human urine was collected from four healthy male and female volunteers who had no history of UTI or antibiotic use in the prior 2 months. The urines were pooled, filter sterilized, stored at 4°C, and used the following day. Overnight cultures of *E. coli* 83972 were grown in pooled human urine or morpholinepropanesulfonic acid (MOPS) minimal medium supplemented with 0.2% glucose until reaching exponential phase and then used for inoculation of 50 ml urine or MOPS to an optical density at 600 nm (OD_{600}) of 0.05. The cultures were grown at 37°C and 130 rpm, and 5-ml samples for isolation of RNA were extracted from three individual cultures at mid-exponential phase (corresponding to OD_{600} values of approximately 0.4 and 0.5 in urine and MOPS, respectively). Extracted samples were immediately mixed with 2 volumes of RNAprotect bacterial reagent (QIAGEN AG), incubated for 5 min at room temperature to stabilize the RNA, and centrifuged. The pellets were then stored at −80°C.

RNA isolation and cDNA labeling. Total RNA was isolated using an RNeasy mini kit (QIAGEN AG). Eluted RNA samples were treated with DNase I and repurified using RNeasy mini columns. The quality of the total RNA was examined by agarose gel electrophoresis and by measuring the absorbance at 260 and 280 nm. Purified RNAs were precipitated with ethanol and stored at −80°C until further use. Conversion of RNAs to cDNAs and microarray analysis were performed according to the protocols in GeneChip expression analysis technical manual 701023, revision 4 (Affymetrix, Inc., Santa Clara, CA).

DNA microarray hybridization. GeneChip *E. coli* Genome 2.0 arrays (Affymetrix) were used for hybridization of the labeled cDNAs. In total, 15 samples were hybridized; three chips were hybridized with samples from *E. coli* 83972 grown in MOPS in triplicate, three chips were hybridized with cells grown in pooled human urine in three individual flasks, and nine chips were hybridized with samples obtained in triplicate from three individual patients. Hybridization, washing, and staining were performed according to GeneChip expression analysis technical manual 701023, revision 4 (Affymetrix), and the microarrays were scanned using a GeneChip Scanner 3000. The GeneChip *E. coli* Genome 2.0 array contains probe sets to detect transcripts from the K-12 strain and three pathogenic strains of *E. coli*. The microarray includes approximately 10,000 probe sets for all 20,366 genes present in the K-12 (MG1655), CFT073 (uropathogenic), EDL933 (enterohemorrhagic), and O157:H7-Sakai (enterohemorrhagic) strains. Due to the high degree of similarity between the *E. coli* strains, whenever possible a single probe set is tiled to represent the equivalent ortholog in all four strains.

Data analysis. Array normalization and expression value calculation were performed using the DNA-Chip Analyzer (dChip) 1.3 software program (http://www.dchip.org/) (30). The invariant set normalization method (31) was used to normalize arrays at the probe cell level to make them comparable, and the model-based (perfect match/mismatch) method was used for probe selection and computing expression values. These expression levels were used with standard errors as measurements of accuracy, which were subsequently used to compute 90% confidence intervals of changes in two-group comparisons. Four different comparisons were performed. (i) In the first comparison, the three arrays hybridized with samples from *E. coli* 83972 grown in MOPS were used as the baseline for the calculation of changes (*x*-fold) in *E. coli* 83972 obtained from each of the three patients (in triplicate). (ii) In the second comparison, the calculation of changes in each of the three patients was performed in the same way as in the first comparison, but with the three arrays hybridized with samples from *E. coli* 83972 grown in vitro in pooled human urine as the baseline. (iii) In a third comparison, the three arrays obtained from samples grown in MOPS were used as a baseline for the calculation of changes in samples grown in pooled human urine. (iv) In the fourth comparison, the calculation of changes in the three patients treated as one sample (nine chips) was performed, using the MOPS arrays as the baseline.

Whether a change in expression of a gene observed between two different chips can be considered significant or not is not solely dependent on the magnitude of the change but also depends on the absolute signals on the two chips. A large up-regulation of a gene has to have a signal that is present and high on the sample chip but not on the baseline chip, while in the case of a small up-regulation (a small change closer to 1.0-fold), the signal has to be present and high on both the sample and baseline chips, and vice versa for down-regulated genes. Therefore, in our analysis, we have only considered an up- or down-regulation to be significant if it meets these criteria, i.e., for a small change (1.4- to 1.9-fold) to be considered significant, the signal has to be present and high on all three baseline arrays as well as the three sample arrays. Moreover, the comparison criteria were carefully chosen to make sure that the empirical false discovery rate (FDR) was kept low ($<$1.8% for all comparisons in our study). The estimation of FDR has become widely accepted as appropriate (14), and it has been argued that the FDR is a more natural scale to work on rather than the *P* value (38). In dChip, estimations of the percentages of genes identified by chance, or empirical FDRs (3), can be performed by permutation. Permuting our samples randomly 200 times resulted in FDRs between 0.1% and 1.7%, or 1 to 23 false-positive genes, for the different comparisons.

RT-PCR. Reverse transcription-PCR (RT-PCR) was performed to confirm the DNA microarray gene expression data. Total RNA was isolated exactly as described above and treated with DNase I to remove any traces of DNA. RNAs were converted to cDNAs by using SuperScript II (Invitrogen). cDNAs were used directly as templates for PCR, and a negative control of the RNA sample (not converted to cDNA) was run in parallel to confirm that all DNA had been removed in the earlier step. The total number of cycles used for PCR ranged from 12 to 30. RT-PCR products were examined by agarose gel electrophoresis.

The following primers were used in RT-PCR and PCR: for *papA*, 620 (5′-GTG AAGTTTGATGGGGCGACC) and 621 (5′-CGCAACTGCTGAGAAAGCA CC); and for the 16S rRNA gene, 622 (5′-CGGATTGGAGTCTGCAACTCG) and 623 (5′-CACAAAGTGGTAAGCGCCCTC).

Growth conditions for iron experiments with *E. coli* 83972 grown in urine. Overnight cultures of *E. coli* 83972 were grown in pooled human urine (as described above) without additives or supplemented with either 200 μM of the iron chelator 2,2-dipyridyl or 10 μM $FeCl_3$. The overnight cultures were used for inoculation of 30 ml urine containing the corresponding concentration of iron to an OD_{600} of 0.05. The cultures were grown at 37°C and 130 rpm in triplicate, and the OD_{600} was measured every hour until all cultures had reached stationary phase.

Microarray data accession number. The supporting microarray data have been deposited in ArrayExpress (http://www.ebi.ac.uk/arrayexpress) with accession number E-MEXP-584.

RESULTS

Patients and sampling of RNA. *E. coli* 83972 was isolated from three patients who had been colonized deliberately with the strain for prophylactic reasons. All three were long-term carriers of the strain, i.e., all patients had carried it for at least 6 months, and one of them had carried it for over 2 years, and none of the patients experienced any UTI-related symptoms during the entire infection period. The patient samples were confirmed monocultures of 83972, and all patients showed significant bacteriuria ($>10^8$ CFU/ml), providing enough bacteria for extraction and purification of RNA with no need for pooling. Thus, a single patient sample yielded enough RNA for three technical replicates.

Global gene expression of *E. coli* 83972 in patients. The genomic expression profiles of *E. coli* 83972 samples obtained from three infected patients (Pat1, Pat2, and Pat3) were compared with those for in vitro *E. coli* 83972 growth in MOPS-glucose medium and pooled human urine. The GeneChip *E. coli* Genome 2.0 array was employed, which contains probe sets for all genes present in *E. coli* strains MG1655, CFT073, EDL933, and O157:H7-Sakai. In total, of 8,716 *E. coli* transcripts, the data analysis resulted in 2,668, 1,452, and 2,947 significantly changed genes for Pat1, Pat2, and Pat3 compared with *E. coli* 83972 grown in MOPS; the corresponding numbers of genes compared with *E. coli* 83972 grown in vitro in urine were 2,205, 1,273, and 1,515, respectively; 2,461 genes were significantly changed in vitro in urine compared with growth in MOPS; and 1,115 genes were significantly changed in all three patients (treated as one single sample group) compared with growth in MOPS.

Overall, of 8,716 genes, 1,892 genes (22%) were expressed at significantly higher levels in any of the three patient samples, and 1,399 genes (16%) were significantly down-regulated in any patient sample compared with *E. coli* 83972 grown in urine in vitro (among these were 570 and 431 CFT073 transcripts, respectively). The corresponding numbers for up- and down-regulated genes for patients compared with MOPS-grown cells were 2,209 genes (25%) and 2,638 genes (30%), respectively (among these were 644 and 955 CFT073 transcripts, respectively). Figure 1 shows the distributions of all significantly changed genes among the three patients; of the genes up-regulated in all three patients (230 and 122 genes compared with growth in MOPS and urine, respectively), 43 genes were identical for both media (Table 1). Of the down-regulated

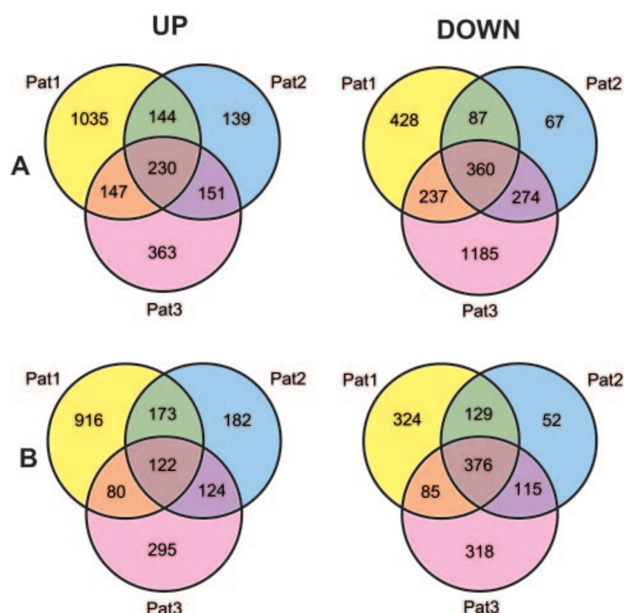

FIG. 1. Venn diagrams showing numbers of significantly up- and down-regulated genes in *E. coli* 83972 in three patients (Pat1, Pat2, and Pat3) compared with those in (A) MOPS-glucose and (B) in vitro urine.

genes, 117 were repressed compared with growth in both urine and MOPS.

Comparison of the gene expression of *E. coli* 83972 in the three patients revealed that Pat1 and Pat3 had larger numbers of significantly changed genes than did Pat2, both compared with growth in urine in vitro and with growth in MOPS (Fig. 1). In total, the expression of 539 genes was seen to be significantly changed (up- or down-regulated) in all three patients compared with growth in vitro in urine. Figure 2 displays the changes in these 539 genes for the different patients and reveals that the majority of the genes (92.4%) were regulated in a similar way: 22.6% and 69.8% were up- and down-regulated, respectively, in all patients; the remaining 7.6% (or 41 genes) showed up-regulation in one patient and down-regulation in another.

Functional analysis of MG1655 transcripts of *E. coli* 83972 in patients. Classification of the significantly changed MG1655 genes into functional groups (57, 58) revealed which groups or categories of genes were affected the most in our patients compared with *E. coli* 83972 grown in MOPS and in vitro in urine. Of a total of 4,070 MG1655 transcripts, 92 and 239 genes showed up- and down-regulation, respectively, in all three patients compared with growth in vitro in urine (see Table S1A in the supplemental material); the corresponding numbers compared with growth in MOPS were 116 and 248 genes (see Table S1B in the supplemental material). The functional analysis revealed that the groups most affected in our patients were those including genes involved in energy production and conversion and amino acid transport and metabolism. The groups including genes involved in inorganic ion transport and metabolism and in nucleotide transport and metabolism were among the most up- and down-regulated, respectively, in the patients compared with growth in both MOPS and urine.

TABLE 1. Genes significantly up-regulated in *E. coli* 83972 in all three patients compared with growth in both MOPS and in vitro urine[a]

Gene	Code	Function or product	Change (fold) in expression compared with that in MOPS			Change (fold) in expression compared with that in urine		
			Pat1	Pat2	Pat3	Pat1	Pat2	Pat3
narH	c1686	Respiratory nitrate reductase 1, beta subunit	9.9	5.8	9.9	53.4	258.7	383.8
fdnI	b1476	Formate dehydrogenase N, gamma subunit, nitrate inducible	10.1	3.1	3.3	37.9	62.2	15.9
narJ	b1226	Nitrate reductase 1, assembly function	11.1	6.6	9.1	96.7	59.1	177.0
yoaG	b1796	Hypothetical protein	2.8	9.5	23.3	12.5	55.9	229.8
ytfE	b4209	Hypothetical protein	2.3	10.9	13.6	9.7	53.2	94.8
narK	b1223	Nitrite extrusion protein	5.6	8.7	2.6	24.9	51.5	11.3
fdnG	ECs5443	Formate dehydrogenase N, alpha subunit, nitrate inducible	6.4	2.7	2.8	80.2	39.9	35.2
yeaR	b1797	Putative tellurite resistance protein	9.5	82.4	90.3	9.5	36.9	81.4
narG	Z2001	Nitrate reductase 1, subunit	8.0	3.8	5.6	71.7	32.2	54.5
ydfZ	b1541	Hypothetical protein	9.2	5.2	4.5	38.5	24.2	27.4
Z0893	Z0893	Putative glutamate mutase, subunit E	139.8	79.7	57.6	23.6	22.9	17.3
narI	b1227	Nitrate reductase 1, gamma subunit	7.0	6.0	8.0	23.3	21.5	29.1
narH	b1225	Nitrate reductase 1, Fe-S (beta) subunit	10.6	5.8	8.3	32.1	21.3	29.6
metA	b4013	Homoserine transsuccinylase	4.6	12.7	2.0	4.8	18.1	2.2
yqgD	b2941	Hypothetical protein	3.5	4.7	7.1	12.0	16.9	32.9
hmpA	b2552	Dihydropteridine reductase, ferrisiderophore reductase activity	6.2	22.2	24.1	3.6	16.0	28.3
nirB	c4141	Nitrite reductase [NAD(P)H] large subunit	2.8	1.9	1.6	18.2	14.3	13.2
fdnH	b1475	Formate dehydrogenase N, beta subunit, nitrate inducible	6.5	2.9	3.2	28.0	14.0	13.9
hcp	b0873	HCP	4.8	2.5	4.7	20.2	13.5	64.6
yhcP	b3240	Putative membrane protein, transport	5.6	3.5	5.0	17.9	10.5	16.5
cspA	b3556	Cold shock protein 7.4, transcriptional activator of *hns*	6.2	3.0	2.9	23.1	10.2	12.5
ygbA	b2732	Hypothetical protein	2.0	5.7	11.3	3.2	9.6	921.0
gntK	b3437	Gluconate kinase 2 in GNT I system, thermoresistant	5.8	2.3	5.6	10.6	7.4	10.3
yhcR	b3242	Hypothetical protein	9.9	7.3	13.6	9.7	7.2	17.5
hcr	b0872	NADH oxidoreductase for HCP	3.7	3.6	4.6	5.7	6.7	9.3
ryfA	c3046	Small RNA	1.5	3.5	5.0	2.3	6.0	8.9
gltB	c3973	Glutamate synthase (NADPH) large chain precursor	1.6	2.2	1.6	3.3	5.6	4.2
ndh	c1382	NADH dehydrogenase	4.1	2.3	3.7	8.1	5.3	9.5
ydcX	b1445	Hypothetical protein	1.7	1.7	2.9	5.1	5.3	10.0
citB	b0620	Response regulator in two-component regulatory system with DpiB	2.8	2.1	1.8	5.5	4.6	3.6
dsdA	c2901	D-Serine dehydratase	7.8	2.4	8.3	14.2	4.6	16.9
yhcQ	b3241	Putative membrane-located multidrug resistance protein	6.9	2.9	5.8	11.8	4.5	10.2
ogt	b1335	*O*-6-Alkylguanine-DNA/cysteine-protein methyltransferase	1.7	2.3	4.9	2.4	4.0	8.9
Z1954	Z1954	Putative Na^+/H^+ exchanger, YcgO	5.2	2.8	2.3	8.2	3.7	3.0
tehB	b1430	Tellurite resistance protein	2.9	3.5	4.9	2.2	3.0	4.8
yhcN	b3238	Hypothetical protein	3.6	3.2	4.6	3.0	2.9	4.7
grxA	b0849	Glutaredoxin 1 redox coenzyme, ribonucleotide reductase	9.9	4.4	6.1	5.9	2.7	4.0
fhuC	b0151	ATP-binding component of hydroxamate-dependent iron transporter	5.0	4.4	4.1	2.1	2.5	2.1
fepE	c0674	Ferric enterobactin transport protein FepE	3.8	3.5	2.7	2.6	2.4	1.8
ydhC	b1660	Putative transport protein	4.4	3.8	5.2	3.0	2.4	3.5
evgA	b2369	Putative positive transcription regulator (sensor EvgS)	2.4	1.8	1.5	2.0	2.0	2.0
nlpI	b3163	Lipoprotein believed to be involved in cell division	2.2	1.8	2.0	2.0	1.7	2.4
Z4919	Z4919	Putative ATP-binding protein of ABC/hemin transport system	5.4	4.6	4.6	1.6	1.5	1.5

[a] The genes are ordered from the highest to lowest change in Pat2 compared with growth in urine.

Up-regulation of iron acquisition genes. The concentration of soluble forms of iron is very low in the urinary tract and is a growth-limiting factor for bacteria (42, 43, 49). Consequently, iron acquisition plays an important role in bacterial survival and pathogenicity. For strain 83972, nearly all genes involved in iron uptake and transport were significantly up-regulated in all the patients and in human urine in vitro compared with growth in MOPS (see Table S2 in the supplemental material). The energy-transducing TonB-ExbB-ExbD complex is needed for transport of iron across the outer membrane. The three genes encoding these proteins were highly up-regulated in the patients (8- to 19-fold) compared with growth in MOPS. Furthermore, all of the *fep* and *ent* genes, encoding proteins involved in enterobactin synthesis, uptake, and transport, were significantly up-regulated in all patients (3- to 40-fold), and so were the genes in the *fhu* cluster (2- to 14-fold) and *iutA* (9- to 22-fold), encoding proteins responsible for uptake and transport of hydroxamate siderophores.

Many iron acquisition genes are associated with bacterial virulence. The *iucABCD* cluster (responsible for aerobactin uptake and transport) and the *sitABCD* cluster (responsible for iron uptake without siderophores), which has been associated with the virulence of *Salmonella enterica* serovar Typhimurium (20), were both highly up-regulated in all three patients compared with growth in MOPS (3- to 30-fold). The *iroBCDEN* and *chuASTWXYU* genes, which were all up-regulated in the three patients (3- to 40-fold), have previously been connected to the urovirulence of *E. coli* in the mouse model (45, 59). A small regulatory antisense RNA (*ryhB*) involved in iron homeostasis was up-regulated 14- to 18-fold in the patients com-

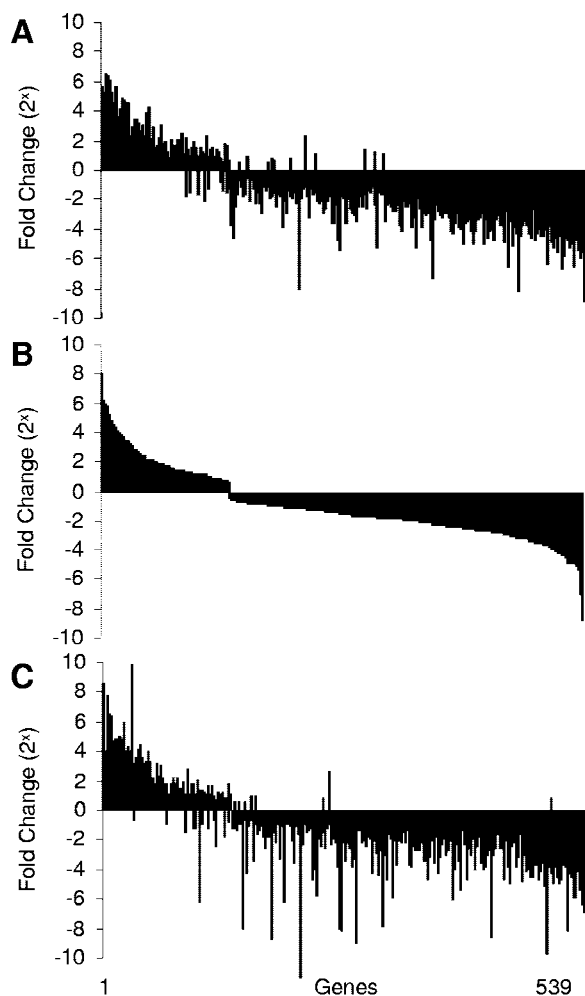

FIG. 2. Comparison of gene regulation in *E. coli* 83972 in the three patients. The 539 genes which were significantly changed in *E. coli* 83972 in all three patients compared with growth in vitro in urine have been ordered from the most up-regulated to the most down-regulated gene in Pat2. Panels A, B, and C represent the changes in the genes in Pat1, Pat2, and Pat3, respectively.

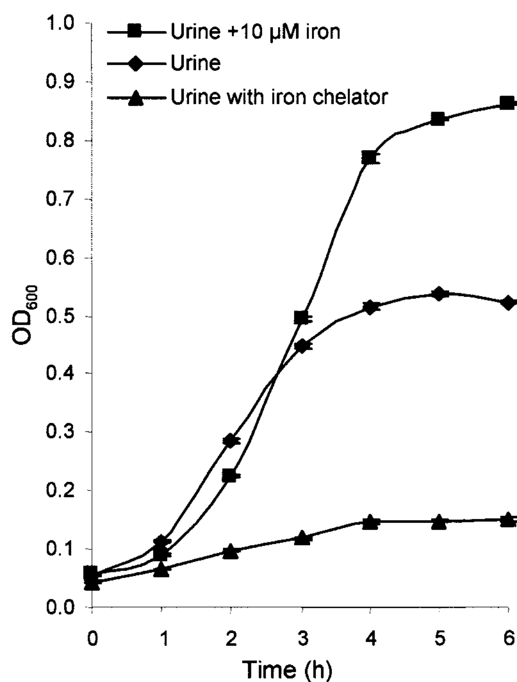

FIG. 3. Growth of *E. coli* 83972 in pooled human urine without additives or supplemented with either 10 μM $FeCl_3$ or 200 μM 2,2-dipyridyl (iron chelator). The values are means of triplicates, and error bars indicate standard deviations (σ_{n-1}).

pared with growth in MOPS. RyhB down-regulates the mRNA levels for genes that are known to be positively regulated by Fur (ferric uptake regulator) (34). The *fur* gene itself showed no change in expression in urine in vitro and in Pat2 and Pat3 compared with growth in MOPS, but it was up-regulated in Pat1 compared with growth in MOPS (1.9-fold).

Taken together, the data indicate that *E. coli* 83972 has numerous genes encoding products that are well suited to cope with an iron-poor environment and that these are highly expressed during growth in the human host. The up-regulation of all of these iron genes underscores that the human urinary tract is an iron-limiting environment. Growth trajectories of *E. coli* 83972 in urine with added iron and in urine with an iron chelator confirmed that the amount of iron in urine is growth limiting and that *E. coli* 83972 employs efficient iron acquisition systems to sustain and enhance growth under iron-limiting conditions (Fig. 3).

Fimbria-encoding genes are down-regulated in humans. The ability of UPEC strains to cause symptomatic UTIs is enhanced by adhesins (e.g., type 1 and P fimbriae) (27), and adherence to urinary tract epithelium enables bacteria to resist removal by urine flow. Our microarray data revealed that half of the *pap* genes were significantly down-regulated in all patients compared with growth in urine: the *papAHCDF* genes were down-regulated 1.9- to 71-fold, and all *pap* genes showed very low signals in all patients. *papA*, encoding the major subunit of P fimbriae, showed a significant 19-fold up-regulation for growth in vitro in urine compared with growth in MOPS but was down-regulated 21- to 24-fold compared with growth in urine for all three patients. While the signals for *fimEAIC* could not be detected in any of the samples due to a large deletion in the *fim* cluster (26), the remaining *fim* genes, *fimB-DFGH*, showed down-regulation for growth in urine compared with growth in MOPS (2- to 5-fold) and were further down-regulated in Pat2 and Pat3 (up to 55-fold) compared with growth in urine. The *foc* cluster encoding F1C fimbriae showed down-regulation in all three patients. For each patient, seven of the nine F1C fimbrial genes were significantly down-regulated (up to 6.1-fold) compared with growth in urine. The *flu* gene, encoding antigen 43, displayed very low signals in all patients, as in the case for growth in MOPS and pooled human urine. None of the newly found (the *auf* cluster) (6) or putative (*yadN*, *ygiL*, *eaeH*, and c4424) fimbrial genes showed any detectable signals (or up-regulation) for any of the patients.

An examination of adherence to human uroepithelial cells of the three patient samples proved negative. Taken together, the data suggest that strain 83972 does not express any fimbriae during colonization of the human urinary tract, and they support earlier observations of the inability of *E. coli* 83972 to adhere to any kind of urinary tract cells (2, 19, 66).

Other virulence factors. Apart from iron acquisition and fimbria-encoding genes, many other genes have been shown to be or are believed to be important for the virulence of UPEC strains (e.g., genes encoding hemolysin, lipopolysaccharides, and motility) (21). The pore-forming hemolysin (HlyA) is considered an important virulence factor in *E. coli* extraintestinal infections, such as those of the upper urinary tract. The genes determining the synthesis, activation, and transport of hemolysin, *hlyCABD*, showed no change in expression in Pat1 and Pat2 but significant down-regulation in Pat3.

None of the genes encoding proteins involved in the biosynthesis of lipopolysaccharides were up-regulated in any of the three patients compared with growth in MOPS; the genes involved in the enterobacterial common antigen biosynthesis pathway (*rffACDGHMT*, *wecBF*, and *rfe*) showed no change or down-regulation; the genes involved in colanic acid biosynthesis (*cpsB*, *gmd*, *fcl*, and *ugd*) were all down-regulated in two or three patients; and the genes involved in the synthesis of lipid A (*lpxBHKP*, *kdsABC*, *htrB*, and *kdtA*) were down-regulated or showed no change. The *kpsEDCS* genes, involved in the transport of polysaccharide to the cell surface, were all down-regulated in Pat2 and -3. The *rcsA* gene, encoding a positive regulator of capsular polysaccharide synthesis, was down-regulated 3.5- to 15-fold in the patients. The *rfaH* gene is another virulence-associated gene which was significantly down-regulated in Pat2 and -3 (1.7- and 2.5-fold, respectively). RfaH is a global regulator which modifies the expression of several virulence factors, and disruption of the *rfaH* gene in uropathogenic *E. coli* has been shown to result in a significant decrease in virulence (37).

The *sat* gene, encoding the secreted autotransporter toxin (Sat) of uropathogenic *E. coli*, showed no change in any of the patients compared with growth in MOPS (16).

The *proP* gene, associated with urovirulence of *E. coli* grown in human urine and colonization of the murine urinary tract (8), was down-regulated in all patients (1.5- to 2.3-fold) and showed no change in human urine.

The *guaA* and *argC* genes have previously been identified as important genes for uropathogenesis (44). *guaA* was down-regulated 3.4- and 3.0-fold in Pat2 and -3, respectively, and up-regulated in urine (1.9-fold), while no change was observed in Pat1. *argC* was up-regulated in Pat1 and -2 (7.3- and 4.3-fold, respectively), while no change was observed in Pat3 or for in vitro growth in urine.

E. coli 83972 carries the virulence-associated *fyuA* gene (10), encoding the yersiniabactin receptor protein. The *fyuA* gene shares 99.9% amino acid identity with c2436 (encoding a putative pesticin receptor precursor), which was highly up-regulated in Pat2 (24.5-fold), up-regulated in Pat3 (2.9-fold), and showed no change in Pat1 or for growth in vitro in urine.

iha occurs frequently among uropathogenic *E. coli* strains, and the Iha adhesin has recently been shown to be a virulence factor in murine urinary tract infections (23). The *iha* gene was significantly up-regulated 2.9- to 3.8-fold in all patients and 14.1-fold in vitro in urine.

Although flagella have been shown to assist *Proteus mirabilis* in colonization of the urinary tract (1), this has never been proven for *E. coli*, and in agreement with the results from a transcriptome study of UPEC in mice (49), none of the genes encoding flagellar proteins (*flg*, *flh*, *fli*, *motAB*, *ycgR*, and *yhiH*) were up-regulated in any of the patients or with growth in urine in vitro (a few of the genes showed down-regulation in the patients). Two recent studies demonstrate that flagella are not essential or absolutely required for virulence of UPEC strains but seem to contribute to the fitness of these strains (28, 65).

Taken together, most virulence factors seemed to be down-regulated or not expressed in our three patients, with the exception of the yersiniabactin-encoding *fyuA* gene and the adhesin-encoding *iha* gene; whether the products of these genes are translated and functional in 83972 remains to be investigated.

Nitrate-rich environment in patients. Human urine contains significant amounts of nitrate (62). This was reflected in the up-regulation of genes encoding nitrate reductase and nitrate-inducible formate dehydrogenase N (Table 1). Nitrate reductase 1 is expressed when levels of nitrate in the environment are high and functions anaerobically as a terminal electron acceptor, forming a respiratory chain together with formate dehydrogenase. The three genes encoding the different subunits of the reductase, *narIGH*, were highly up-regulated in all three patients (Table 1), as were the genes encoding formate dehydrogenase, *fdnGHI* (Table 1). The genes encoding NarJ, essential for nitrate reductase activity, and NarK, a nitrite extrusion protein, were also up-regulated in all three patients (Table 1).

The genes encoding the nitrite reductase (which is present in *E. coli* in high concentrations of nitrate, with the probable metabolic role of detoxifying nitrite), *nirB* and *nirD*, were up-regulated in all patients (7.5- to 18.2-fold) compared with growth in urine. *nrfA*, which encodes the other nitrite reductase of *E. coli*, which is activated at low nitrate concentrations, showed significant down-regulation in two of the patients.

Two highly up-regulated genes in all patients were *hcp* and *hcr*, encoding the hybrid cluster protein (HCP) and its NADH oxidoreductase, respectively (Table 1). These proteins are known to be produced during anaerobic growth in the presence of nitrite or nitrate (63). Due to its regulation, HCP is thought to be involved in nitrite or nitrate reduction, accomplishing an as yet unidentified reaction in the nitrogen cycle (11). The corresponding protein in *Salmonella enterica* exhibits a role in pathogenesis, and a previous study indicated that nitric oxide is a good candidate as the substrate of HCP (25). Recently, *hcp* was found to be induced in *E. coli* exposed to nitric oxide under both aerobic and anaerobic conditions (12).

Taken together, the up-regulation of the genes involved in nitrate metabolism and nitrite detoxification reveals that strain 83972 is exposed to a nitrate-rich environment and efficiently uses nitrate for respiration.

Up-regulation of genes involved in NO protection and metabolism. High concentrations of NO are produced by activated macrophages to fight prokaryotic pathogens and parasites, and this NO production plays a key role as one of the immune system's weapons against pathogens. *E. coli* contains proteins that are proposed to metabolize NO and thereby provide protection for the cells. Also, a recent study demonstrated a correlation between NO tolerance and the virulence of UPEC strains (55). Many key genes involved in NO protection were highly up-regulated in our three patients (Table 1). NO is removed and detoxified by flavohemoglobin, encoded by the *hmpA* gene, and the flavorubredoxin encoded by *norV*

TABLE 2. Top 50 genes expressed by *E. coli* 83972 in vivo in patients

Gene	Code	Function or product	Signal intensity[a]	Member of top 50 genes expressed in urine	Member of top 50 genes expressed in MOPS
rplE	b3308	50S ribosomal subunit protein L5	3,788	+	+
rrfH	b0205	5S rRNA	3,554	+	+
t44	b4414	Small toxic membrane polypeptide	3,508	+	+
rplJ	b3985	50S ribosomal subunit protein L10	3,493	+	+
csrB	b4408	CsrB regulatory RNA	3,462	+	+
cspC	b1823	Cold shock protein	3,445		
rpsK	b3297	30S ribosomal subunit protein S11	3,437	+	+
ahpC	b0605	Alkyl hydroperoxide reductase	3,418	+	
rrsG	b2591	16S rRNA	3,244		+
rpsH	b3306	30S ribosomal subunit protein S8	3,222	+	+
tufA	b3339	Protein chain elongation factor EF-Tu	3,185	+	+
rpsD	b3296	30S ribosomal subunit protein S4	3,182	+	+
rplX	b3309	50S ribosomal subunit protein L24	3,166	+	+
rpoA	b3295	RNA polymerase, alpha subunit	3,128	+	+
acpP	b1094	Acyl carrier protein	3,068	+	
ompC	c2758	Outer membrane protein C precursor	3,048	+	+
rplN	b3310	50S ribosomal subunit protein L14	3,043	+	+
rpsM	b3298	30S ribosomal subunit protein S13	3,037	+	+
rplM	b3231	50S ribosomal subunit protein L13	3,030	+	+
ssrA	b2621	tmRNA[b]	2,989		+
infC	Z2747	Initiation factor IF-3	2,981		+
rplC	b3320	50S ribosomal subunit protein L3	2,977		+
rnpB	b3123	RNase P, RNA component	2,975		+
rpsG	b3341	30S ribosomal subunit protein S7	2,933	+	+
rpmJ	b3299	50S ribosomal subunit protein L36	2,908		+
eno	b2779	Enolase	2,894		
rhoL	b3782	Rho operon leader peptide	2,862		
rplK	b3983	50S ribosomal subunit protein L11	2,805	+	+
yfiD	b2579	Stress-induced alternate pyruvate formate-lyase	2,797		
lpp	b1677	Murein lipoprotein	2,767	+	+
folE	b2153	GTP cyclohydrolase I	2,742	+	
aceE	b0114	Pyruvate dehydrogenase	2,739		+
rplL	b3986	50S ribosomal subunit protein L7/L12	2,719	+	+
rpsB	b0169	30S ribosomal subunit protein S2	2,719		+
c4310	c4310	Hypothetical protein	2,714		
csrC	b4457	CsrC regulatory RNA	2,712		
rplO	b3301	50S ribosomal subunit protein L15	2,699	+	+
ptsH	b2415	PTS family Hpr protein	2,681		
rplF	b3305	50S ribosomal subunit protein L6	2,677	+	+
rpmB	b3637	50S ribosomal subunit protein L28	2,674		
gapA	b1779	Glyceraldehyde-3-phosphate dehydrogenase A	2,665		
rpsL	b3342	30S ribosomal subunit protein S12	2,665		+
rplU	b3186	50S ribosomal subunit protein L21	2,654		
rpsN	b3307	30S ribosomal subunit protein S14	2,648		+
hupA	b4000	DNA-binding protein HU-alpha (HU-2)	2,648	+	
gpmA	b0755	Phosphoglyceromutase 1	2,645	+	
fusA	b3340	GTP-binding protein chain elongation factor EF-G	2,620	+	+
rpsJ	b3321	30S ribosomal subunit protein S10	2,616		+
yobF	b1824	Hypothetical protein	2,565		
rpsS	b3316	30S ribosomal subunit protein S19	2,564		+

[a] Signal intensities were calculated from all nine patient arrays treated as a single sample group. Values are model-based expression indexes calculated by dCHIP.
[b] tmRNA, transfer messenger RNA.

reduces NO; the *hmpA* gene was up-regulated in all three patients (Table 1), and the *norV* gene was significantly up-regulated in all three patients (4.2- to 8.6-fold) compared with growth in MOPS. Recently, a new gene was demonstrated to be involved in protection against NO; *E. coli* treated with NO under anaerobic growth conditions showed increased levels of *ytfE* mRNA, and mutants were more sensitive to NO than the wild type (24). This gene was up-regulated up to 95-fold in the patients (Table 1).

The tellurite resistance genes *tehAB* have been shown to be induced when *E. coli* is exposed to NO (24) or the antibacterial compound 4,5-dihydroxy-2-cyclopenten-1-one (39). The *tehB* gene was up-regulated in all three patients (Table 1), and *tehA* was up-regulated 2.9- and 3.6-fold compared with growth in MOPS in Pat2 and Pat3, respectively. Furthermore, the putative tellurite resistance gene *yeaR* showed significant up-regulation in all patients (Table 1). The *yeaR* gene (b1797) has been shown to be induced by nitrate, together with the *yoaG* gene (b1796) (15), which also showed significant up-regulation in all patients (Table 1).

ahpC, the gene encoding alkyl hydroperoxide reductase, has also been suggested to contribute to resistance against reactive

nitrogen species (7). This gene was highly expressed and displayed the eighth highest signal in the patients (Table 2).

The *yhaO* gene was up-regulated 2.8- to 9.9-fold in the patients. This gene, encoding a hypothetical transport protein, was one of the most up-regulated genes in response to nitrosative stress induced by *S*-nitrosoglutathione (12). Other genes that showed up-regulation in response to nitrosative stress (12) were up-regulated in our patients: two hypothetical proteins encoded by *yhaN* and *yhaM* were up-regulated 1.9- to 14-fold in all patients, and six genes involved in methionine biosynthesis or regulation, *metABFJNR*, were up-regulated 1.6- to 4.6-fold and 3.2- to 13-fold in Pat1 and -2, respectively. Taken together, the data suggest that 83972 is perhaps not defenseless and could well be able to resist oxidative bursts from neutrophils.

High expression of ribosomal genes in the human urinary tract. Thirty of the 50 highest expressed genes of *E. coli* 83972 in patients were ribosomal proteins or those involved in transcription/translation (Table 2). Of 55 ribosomal genes and three rRNA species, 46, 28, 52, 36, and 42 genes belonged to the top 100 most highly expressed genes in MOPS, urine, and Pat1, -2, and -3, respectively, and 57 of 58 genes belonged to the 5% of genes with the highest expression on all arrays. The high expression of ribosomal genes reveals a high growth rate, indicating fast growth of *E. coli* 83972, particularly in Pat1 and MOPS.

Low oxygen levels in the human urinary tract. Our results suggest that strain 83972 uses nitrate respiration, which is indicative of limited oxygen availability. In line with this notion, the expression of the *cyoABCD* genes, encoding cytochrome *o* oxidase (used under high-oxygen growth conditions), was down-regulated 5.3-, 14.3-, 13.7-, and 13.6-fold (average values of significant changes in the three patients), respectively; the *cyoE* gene, essential for the catalytic function of the cytochrome complex, was down-regulated 16.7-fold. The genes encoding the cytochrome *d* complex, *cydAB*, which is maximally expressed under microaerobic conditions, were moderately up-regulated (1.6- and 1.8-fold) in the patients. *arcA* and *fnr*, the key regulators of respiration, showed no change in the patients or in urine in vitro. Taken together, this reveals that 83972 experiences a limited amount of oxygen and that the human urinary tract seems to be a microaerobic environment.

Up-regulation of genes involved in carbohydrate and amino acid transport and metabolism. A substantial part of the genes involved in carbohydrate and amino acid transport and metabolism was up-regulated in our patients. However, only a few genes were up-regulated in all three patients, revealing that *E. coli* 83972 encounters somewhat different nutrients in urine depending on the patient (see Tables S1 and S3 in the supplemental material). D-Serine is excreted in large amounts in human urine (5), and it has been suggested that D-serine catabolism provides a growth advantage in the urinary tract since it is a good nitrogen and carbon source (40). The gene encoding D-serine deaminase, *dsdA*, which converts D-serine to pyruvate, was up-regulated in all three patients (2.4- to 8.3-fold) compared with growth in MOPS (Table 1), together with the D-serine permease gene, *dsdX*, which was up-regulated 7.4- to 7.8-fold.

The microarray data did not reveal any up-regulation of genes involved in starvation; the carbon and phosphate starvation genes *astC*, *slp*, and *phoP* were significantly down-regulated in the patients compared with growth in MOPS-glucose (3.9- to 5.3-fold). The up-regulation of carbohydrate and amino acid genes, together with the down-regulation of starvation genes, indicates that *E. coli* 83972 does not experience any starvation and is able to efficiently utilize the nutrients available in urine.

Other up-regulated genes. Trimethylamine *N*-oxide (TMAO) is naturally found in urine (64) and is formed from dietary choline, with eggs, liver, and soybeans being the main sources; when marine fish is a dietary component, several hundred milligrams of TMAO may be excreted. The *torA* and *torC* genes, encoding TMAO reductase I, a terminal electron acceptor, were significantly up-regulated (5.4- to 15-fold) in all three patients.

The *ycdO* gene (b1018), which has been shown to be induced by a low pH (54), was highly up-regulated in the patients compared with growth in MOPS (6.6- to 20-fold). The two adjacent genes, b1016 (*ycdN*, encoding a hypothetical protein) and b1017 (encoding a high-affinity iron permease), were both up-regulated 3.2- to 18-fold in the three patients. The iron-dependent regulation of *ycdN-ycdOB*, revealed in a global gene regulation study, suggested that this cluster might represent a novel iron transporter (35). The *ycdB* gene was up-regulated 5.1- and 8.5-fold in Pat2 and -3, respectively, but showed no change in Pat1.

Verification of microarray results. RT-PCR was performed to verify the transcript levels of selected genes. *papA* was up-regulated 19-fold in urine in vitro but showed very low signals in all patients and in MOPS. *papA* could not be detected in the samples from MOPS and Pat1 to -3, not even after 30 cycles of PCR, while *papA* was detected in all three in vitro urine samples and visualized as strong bands on an agarose gel. 16S rRNA was used as a normalizing internal standard and was detected with the same intensity in all samples.

The expression levels of the *fim* genes revealed the sensitivity of the microarrays. Sequencing of the *fim* cluster of *E. coli* 83972 has revealed that a large part of the cluster is deleted in *E. coli* 83972, i.e., 4.25 kb is deleted between *fimB* and *fimD*, resulting in the complete absence of *fimEAIC* and leaving only *fimF*, *fimG*, and *fimH* unaffected (26). The signal from *fimEAIC* was very low (absent call) and varied between −9 and 84, with averages of 17, 19, 50, 22, and 42 for growth in MOPS, urine, and Pat1, -2, and -3, respectively. The signals of *fim BDFGH* varied between 5 and 1,209, with averages of 1,000, 275, 448, 122, and 25 for growth in MOPS, urine, and Pat1, -2, and -3, respectively. The signal levels (i.e., absent/present calls) of the different *fim* genes on the arrays corresponded well with the actual presence of *fim* genes in *E. coli* 83972.

To verify the up-regulation of iron acquisition systems in *E. coli* 83972 grown in urine (in vitro and in vivo), growth in pooled human urine with different iron concentrations was performed. The growth trajectories and higher final cell densities with added iron support the conclusion derived from the microarray data that iron availability in urine is limiting for the growth of *E. coli* 83972 (Fig. 3).

DISCUSSION

DNA microarray-assisted functional genomics provides the global expression profile of a genome. The bacterial transcrip-

tome is a dynamic entity that reflects the organism's immediate, ongoing response to its environment. Microarray expression profiling therefore provides a comprehensive snapshot of the transcriptome. The expression profile discloses how the bacterium adapts to an environmental niche. Adaptation to a given host environment is an extremely important parameter and underlies the capacity of an infectious agent to persist in a host.

Contamination with nucleic acids from the host and from other bacteria makes analysis of bacterial global gene expression in humans very difficult. To our knowledge, only one other study has addressed global bacterial gene expression in human hosts, i.e., a study of expression profiling of *Vibrio cholerae* isolated from rice water stools of patients (4, 29); in this case, the large flow rate minimized contamination with other bacteria. Transcriptional profiling of *E. coli* 83972 during colonization of the human urinary tract was possible because the human urinary tract is quite amenable to this type of approach. Firstly, because the UT is naturally sterile and, when infected, is almost invariably colonized with a single strain, UTIs can for all practical purposes be considered monocultures of a given bacterium. Also, contamination with host nucleic acids is generally insignificant. Finally, a single urine sample from a human patient with UTI contains sufficient bacterial RNA to permit microarray analysis in triplicate, with no pooling of samples or dilution of gene expression.

E. coli 83972 is a good colonizer of the human bladder but has never been reported to ascend further up the urinary tract. Arguably, the transcriptional profiles of the strains obtained from three patients reflect the unique response of *E. coli* 83972 to the prevailing conditions in the human bladder. Bacterial gene expression is a function of the actual environmental conditions. Our study encompassed three different patients, and the conditions in the urinary tracts of these three persons are inherently somewhat different. It was therefore expected that the expression profiles of *E. coli* 83972 would vary somewhat depending on the host. In effect, this turned out to be true, and the expression of a subset of genes was observed to be affected in one patient but not in others (Fig. 1 and 2), particularly for genes involved in amino acid and carbohydrate transport and metabolism. Consequently, we have chosen to focus on the expression of genes that were affected similarly in all three patients.

The holding capacity of the human bladder is 300 to 500 ml, and the residual urine after micturition amounts to 1 ml (48). For obvious reasons, bacterial adherence is generally considered a pivotal step in the colonization of host tissue surfaces subjected to hydrodynamic flow forces, such as the human bladder. The three primary fimbrial adhesins associated with UT colonization by *E. coli* are type 1, F1C, and P fimbriae. The genome of *E. coli* 83972 contains genes at several fimbria-encoding loci, such as *pap*, *foc*, and *fim* (19). Our array data showed that the expression of none of these was up-regulated in patients. In this respect, it is worth noting that P fimbriae have primarily been implicated in kidney colonization, and strain 83972 colonizes the bladder. Recently, it was seen that in *E. coli* strain CFT073, the *fim* gene cluster was highly expressed in the murine UT (49), while P and F1C fimbriae were expressed in the absence of type 1 fimbriae, in *fim* and *fim/pap* mutants, respectively (50). Here it is important to keep in mind that (i) the conditions in the human and murine UT are not necessarily the same and (ii) strain 83972 and strain CFT073 are quite different: while the first causes asymptomatic bacteriuria, the latter is, by all criteria, a bona fide UPEC strain. The two strains seem to have opted for different strategies in their interplay with the host; while *E. coli* CFT073 has all the hallmarks of an aggressive pathogen, *E. coli* 83972 seems to have a more commensal-like behavior vis a vis the host. Type 1, F1C, and P fimbriae trigger host responses that include cytokine production, inflammation, and exfoliation of infected bladder epithelial cells (36, 46, 67). The inability of strain 83972 to trigger an aggressive host response can be accounted for, at least partly, by its inability to express functional fimbriae. Several other known virulence factors are down-regulated in the human host, such as genes involved in hemolysin and capsule synthesis. However, some virulence factor-encoding genes are up-regulated in patients; for example, genes involved in protection against NO are highly expressed. NO is an ingredient of the oxidative burst that neutrophils use to eliminate bacteria, and this NO-protective gene induction might indicate that *E. coli* 83972 does activate some host defense mechanisms. Also, the sensitivity to NO varies among *E. coli* strains, and UPEC strains are less susceptible to NO than, for example, K-12 strains (55).

It is interesting that the majority of the most highly expressed genes of *E. coli* 83972 in humans encode products that are involved in protein synthesis, such as ribosome components. A similar trend was seen for CFT073 in a mouse model (49). It is well known that the rate of ribosome synthesis is strongly coupled to the growth rate of the cell (33), and genes encoding ribosomal proteins have been shown to be highly expressed during exponential growth in rich media (56). The high expression of ribosomal genes in *E. coli* 83972 suggests a rapid growth rate in the human urinary tract and supports our earlier hypothesis that the strain's excellent, supreme growth properties in human urine explain its ability to successfully colonize the human urinary tract in the absence of functional fimbriae (42). The array data indicate that the excellent growth properties of strain 83972 are due to efficient iron uptake systems combined with microaerobic growth, with respiration of nitrate coupled to degradation of sugar acids and amino acids. Whether other unknown factors play a role remains to be shown. It should be noted that even though the *E. coli* arrays employed in this study included transcripts from the uropathogenic strain CFT073, the whole genome of *E. coli* 83972 has not yet been sequenced, and therefore the possibility that strain 83972 carries unique genes enabling its efficient growth in urine cannot be ruled out at this stage.

Strain 83972 has adapted considerably to a commensal-like way of living in the human bladder, growing very fast in urine and avoiding the host response, which is well reflected in the results of its global gene expression profile presented here. It has had more than 30,000 generations to adapt to this particular environment (42). Most virulence factors, such as those encoding adhesins and capsule, were down-regulated. However, a few virulence-associated genes, such as those encoding products used for protection against NO, potentially produced by macrophages, were up-regulated, indicating that strain 83972 is not defenseless. Meanwhile, the hallmark of the strain seems to be its ability to grow extremely well in human urine

(42), and this faculty is certainly reflected in its gene expression profile.

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